

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant :	Jeffrey Olson et al.	Art Unit :	1637
Serial No. :	09/697,028	Examiner :	Suryaprabha Chunduru
Filed :	October 25, 2000	Conf. No. :	3430
Title :	METHODS FOR GENETIC ANALYSIS OF DNA TO DETECT SEQUENCE VARIANCES		

**Mail Stop Appeal Brief - Patents**

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

**BRIEF ON APPEAL**

**(1) Real Party in Interest**

The Real Party in Interest is Sequenom, 3595 John Hopkins Court, San Diego, CA  
92121-1331

**(2) Related Appeals and Interferences**

There are no related appeals or interferences.

**(3) Status of Claims**

Claims 10-16 are pending and presented in the claims appendix.

Claims 10-16 are rejected under 35 U.S.C. § 102(e).

**(4) Status of Amendments**

All of the amendments filed in this case have been entered.

**(5) Summary of Claimed Subject Matter**

The presently pending claims (10-16) relate to DNA amplification methods that cause differential amplification of two nucleic acid molecules that differ in sequence at a polymorphic site. Thus, a first nucleic acid molecule having a first allele at a polymorphic site is amplified to a greater extent than a second nucleic acid molecule having a second allele at the polymorphic site. Such methods are useful, for example, when one wishes to obtain relatively more copies of one of the two nucleic acid molecules for analysis.

The independent claims require the use of two amplification primers, neither of which hybridize to the polymorphic site. One of the two primers includes a "5' portion which, when incorporated into the amplification product, will upon further amplification yield products that form a stable stem-loop structure, the stem of which is perfectly matched and includes the polymorphic site only when the second nucleotide is present at the polymorphic site, but not when the first nucleotide is present at the polymorphic site". Thus, one of the primers has a 5' region that upon integration into amplification product, will form a stem-loop having a perfectly matched stem containing the polymorphic site when the second allele (second nucleotide) is present at the polymorphic site, but not when the first allele (first nucleotide) is present at the polymorphic site. Since a sufficiently stable stem-loop interferes with further amplification, the formation of a perfectly matched stem in the case of second allele will cause the nucleic acid molecule containing the second allele (the second nucleic acid molecule) to be amplified to a lesser extent than the nucleic acid molecule (the first nucleic acid molecule) containing the first allele. This result is referred to as differential amplification.

**(6) Grounds of Rejection**

Claims 10-16 are rejected under 35 U.S.C. §102(e) as anticipated by U.S. Patent No. 6,326,145.

**(7) Argument**

**The Rejection of Claims 10-16 under 35 U.S.C. §102(e) as anticipated by as anticipated by U.S. Patent No. 6,326,145 Should be Reversed**

The Examiner has rejected claims 10-16 under 35 U.S.C. §102(e) as anticipated by as anticipated by U.S. Patent No. 6,326,145 ("the '145 patent").

**Brief Summary of the Rejection**

The Examiner rejected claims 10-16 under 35 U.S.C. §102(e) as anticipated by the '145 patent". The Examiner, citing Example 1 (col. 12, lines 54-67 and col. 13, lines 1-52) of the '145 patent, argued that the '145 patent discloses a method for achieving differential amplification of two different alleles in a mixture such that a first nucleic acid molecule having a first nucleotide present at a polymorphic site (a first allele) is amplified to a greater extent than a second nucleic acid molecule having a second, different nucleotide present at the polymorphic site (a second allele). The Examiner stated that the '145 patent disclose differential amplification because the fluorescence signal "is proportional to the amount of the target present in the sample". Citing other portions of the '145 patent, the Examiner argued that the '145 patent describes a probe that forms a stem loop only when certain sequence are present in a target nucleic acid molecule that serves as template for extension of the primer.

Appellants' Rebuttal

The '145 patent does not anticipate the present claims because it does not teach differential amplification required by the present claims and because the probes described in the '145 do not include a 5' portion that is incorporated into an amplification product as required by the present claims.

*The '145 Patent*

The '145 patent describes a detection probe ("Scorpion probe") that is capable of forming a stem-loop when a selected sequence is present in a sample. The Scorpion probe includes a signaling system such as a fluorophore and a quencher. The Scorpion probe also includes a template binding region that hybridizes to a template nucleic acid molecule. The Scorpion probe also includes a target binding region that can participate in the formation of the stem of a stem-loop structure if the probe is extended to include a sequence complementary to the target binding region. When the desired template sequence is present in a sample, extension of the Scorpion probe leads to the formation of a stem-loop that prevents the fluorophore of the Scorpion probe from interacting with the quencher of the Scorpion probe, thereby allowing a fluorescent signal to be emitted as an indication that the desired template sequence is present in a sample. As the '145 patent explains, the Scorpion probe is a sensitive means for detecting a particular sequence. The Scorpion probe hybridizes to a sequence within a template nucleic acid molecule *via* the template binding region of the Scorpion probe. Extension of the Scorpion probe on the target will cause the incorporation into the probe of additional sequence. If extension of the Scorpion probe creates a sequence that is complementary to the target binding region already present within the Scorpion probe, then a stem-loop structure forms and a fluorescent signal is emitted. If extension of the Scorpion probe creates a sequence that is NOT complementary to the target binding region already present within the Scorpion probe, then the stem-loop structure does NOT form and NO fluorescent signal is emitted. Thus, it can be seen that Scorpion probe is a detection system, not a primer for differential amplification. In fact, the '145 patent explains (col. 5, lines 51-56) that the methods described are detection methods that are used in conjunction with amplification methods (col. 5, lines 64-65).

*The '145 patent does not teach differential amplification required by the claims*

The method of the present claims results in differential amplification such that a "first nucleic acid molecule is amplified to a greater extent than the second nucleic acid molecule". Thus, the claims describe a method in which "a first nucleic acid molecule having a first nucleotide present at a polymorphic site is amplified to a greater extent than a second nucleic acid molecule having a second, different nucleotide present at the polymorphic site".

The '145 patent simply does not disclose a method for achieving differential amplification of two different nucleic acid molecules. The Examiner has failed to demonstrate that Example 1 (or any other portion) of the '145 patent discloses differential amplification. Example 1 of the '145 patent discloses a PCR amplification reaction that takes place in the presence of a Scorpion probe that serves mechanism for detecting a nucleic acid molecule having a particular sequence, e.g., a target allele. Fig. 13, which depicts the results of the experiment in Example 1, shows that the fluorescent signal produced by the Scorpion probe that matched the target allele increased with time during a PCR amplification reaction. However, contrary to the Examiner's assertion, this is not an indication that differential amplification has occurred. In other words, it does not provide any evidence that a nucleic acid molecule that differed from the target allele at a single polymorphic site, had it been present, would have been amplified to a different degree. Indeed there is no indication that two nucleic acid molecules differing by the nucleotide present at a polymorphic site are even present in the experiment, much less that two different nucleic acid molecules were differentially amplified. Thus, nothing cited by Examiner suggests that the '145 patent teaches differential amplification of two molecules that differ in the nucleotide present at a polymorphic site, as is required by the present claims.

Moreover, the '145 patent appears to teach just the opposite of differential amplification. Example 2 of the '145 patent describes the use of two different Scorpion probes -- one that matches the sequence present in the template nucleic acid molecule and one that does not match the sequence present in the template nucleic acid molecule.<sup>1</sup> Each Scorpion probe was added to a PCR amplification reaction containing the template nucleic acid molecule. As shown in Fig. 14, the matched Scorpion probe (i.e., the one having a target binding region perfectly complementary to a sequence in the template nucleic acid molecule) generated a strong

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<sup>1</sup> Note that instead of there being single probe and two targets differing at a single position, there are two different probes differing at a single position and a single target. This is the mirror image of the normal detection situation.

fluorescent signal while the mismatched Scorpion probe (i.e., the one having a target binding region that is NOT perfectly complementary to a sequence in the template nucleic acid molecule) did not generate a significant fluorescent signal. The '145 patent explains that "both amplifications were equally efficient" (col. 13, lines 58-60). In other words, the mismatch Scorpion probe amplified the target just as well as the matched Scorpion probe. However, only the matched Scorpion probe, which can form a stem loop and thus avoid self-quenching, emitted a fluorescent signal. Thus, the '145 patent itself teaches that the Scorpion probe does not cause differential amplification. If the Scorpion probes were causing differential amplification, the efficiency of the amplification reaction would depend on whether the matched or mismatched Scorpion probe was present in the amplification reaction. Applicants grant that this is a situation with two different probes, not two different templates. However, the experiment shows that there is the same degree of amplification whether or not a Scorpion probe is exactly match to target or is mismatched. For this reason alone it is clear that the '145 patent cannot anticipate the present claims.

*The probes described in the '145 patent do not include a 5' portion that is incorporated into an amplification product*

One of the two primers used in the presently claimed methods includes "a 5' portion which, when incorporated into an amplification product, will upon further amplification yield products that form a stable stem-loop structure..." The Scorpion probe of the '145 patent can form a stem-loop, but is very different. As the '145 patent explains, when the Scorpion probe is used in an amplification system such as PCR, the Scorpion probe includes a blocking moiety (e.g., hexethylene glycol) that is located between the template binding region and the 5' target binding region that prevents the 5' target binding region from being amplified (see col. 2, lines 54-67). It is this non-amplified 5' target binding region that forms the stem of the stem-loop that forms when the proper target sequence is present. As explained above, it is this stem-loop formation that prevents quenching of the fluorescent signal thus allowing a fluorescent signal to be admitted. Thus, the Scorpion probes do not contain a 5' region that is incorporated into the amplification product and forms a stem-loop structure (when a certain target sequence is present), as required by the present claims. For this second, independent reason, it is clear that the '145 patent cannot anticipate the present claims.

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**(8) Conclusion**

For at least the above reasons, the 35 U.S.C. § 102(e) rejection of claims 10-16 should be reversed.

The required fee in the amount of \$250 for the brief fee is submitted herewith via EFS by way of Deposit Account authorization. A Petition for Extension of Time with the appropriate fee is also being submitted herewith. Please apply any other charges or credits to Deposit Account No. 06-1050, referencing Attorney Docket No.: 20751-004001.

Respectfully submitted,

Date: 8 March 2007

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### **Appendix of Claims**

10. A method for biasing a DNA amplification reaction such that a first nucleic acid molecule having a first nucleotide present at a polymorphic site is amplified to a greater extent than a second nucleic acid molecule having a second, different nucleotide present at the polymorphic site, comprising

(a) contacting a sample of DNA comprising at least the first nucleic acid molecule with two amplification primers that hybridize to both the first nucleic acid molecule and the second nucleic acid molecule at locations which flank the polymorphic site such that neither the first primer nor the second primer hybridizes to the polymorphic site, one of the two primers including a 5' portion which, when incorporated into an amplification product, will upon further amplification yield products that form a stable stem-loop structure, the stem of which is perfectly matched and includes the polymorphic site only when the second nucleotide is present at the polymorphic site, but not when the first nucleotide is present at the polymorphic site; and

(b) carrying out an amplification reaction, whereby the first nucleic acid molecule is amplified to a greater extent than the second nucleic acid molecule.

11. The method of claim 10, wherein the DNA is single-stranded DNA.

12. The method of claim 10, wherein the DNA is double-stranded DNA.

13. The method of claim 10 further comprising separately carrying out steps (a) and (b) for each of a plurality of polymorphic sites.

14. The method of claim 10 wherein the sample of DNA comprises mammalian DNA.

15. The method of claim 14 wherein the sample of DNA comprises human DNA.

16. A method for haplotyping a first nucleic acid molecule having a first nucleotide present at a polymorphic site present in a sample of DNA comprising at least the first nucleic

acid molecule and a second nucleic acid molecule having a second, different nucleotide present at the polymorphic site, comprising:

(a) contacting a sample of DNA comprising at least the first nucleic acid molecule with two amplification primers that hybridize to both the first nucleic acid molecule and the second nucleic acid molecule at locations which flank the polymorphic site such that neither the first primer nor the second primer hybridizes to the polymorphic site, one of the two primers including a 5' portion which, when incorporated into an amplification product, will upon further amplification yield products that form a stable stem-loop structure, the stem of which is perfectly matched and includes the polymorphic site only when the second nucleotide is present at the polymorphic site, but not when the first nucleotide is present at the polymorphic site;

(b) carrying out an amplification reaction, whereby the first nucleic acid molecule is amplified to a greater extent than the second nucleic acid molecule to create an amplified DNA sample; and

(c) determining the nucleotide sequence of at least a portion of the DNA present in the amplified DNA sample.

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### **Evidence Appendix**

None

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### **Related Proceedings Appendix**

None